PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/013780	03.12.2004	03.12.2003

International Patent Classification (IPC) or both national classification and IPC
C12N15/82, C12N5/10

Applicant
ICON GENETICS AG

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Mundel, C

Telephone No. +49 89 2399-7314

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2004/013780

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority for the purposes of search.

3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. IV Lack of unity of invention

1. ☐ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ not paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☒ all parts.

   ☐ the parts relating to claims Nos.

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	7-8, 14-18, 20-25, 27, 40
	No:	Claims	1-6, 9-13, 19, 26, 28-39, 41-42
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-13, 19, 26, 28-42
Industrial applicability (IA)	Yes:	Claims	1-42
	No:	Claims	

2. Citations and explanations

   see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

International application No.
PCT/EP2004/013780

Re Item IV
Lack of unity of invention

The common concept underlying the present application, i.e. a process of controlling expression of a plastome-encoded sequence of interest in a plant or in plant cells by externally applying to said plant or to said plant cells a control signal selected from a physical signal or a chemical signal or a source thereof, cannot be considered as novel (see point V-3 below) or inventive (see point V-4 below). Therefore

Re Item V
Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The present application refers to a process of controlling the expression of a plastome-encoded protein of interest in a plant or in plant cells by externally applying to said plant or to said plant cells a control signal selected from a physical signal or a chemical signal. The application also refers to plants or plant cells capable of controlled expression of a plastome-encoded sequence of interest.

2. **Reference is made to the following documents :**

   D1: WO 98/11235 A (CIBA GEIGY AG ;HEIFETZ PETER (US); LEBEL EDOUARD (US); UKNES SCOTT) 19 March 1998 (1998-03-19)
   D2: US-A-5 925 806 (MCBRIDE KEVIN E ET AL) 20 July 1999 (1999-07-20)
   D3: US-A-5 877 402 (CARRER HELAINE ET AL) 2 March 1999 (1999-03-02)
   D4: HEIFETZ P B ET AL: "TRANSGENICS AND BIOTECHNOLOGY CHEMICAL REGULATION OF NUCLEAR AND PLASTID TRANSGENES IN PLANTS" PLANT PHYSIOLOGY, AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ROCKVILLE, MD, US, vol. 114, no. 3, 5 August 1997 (1997-08-05), page 308.
   D5: HEIFETZ P B: "Genetic engineering of the chloroplast" BIOCHIMIE, MASSON, PARIS, FR, vol. 82, 2000, pages 655-666.
   D6: US 2003/009783 A1 (DANIELL HENRY ET AL) 9 January 2003 (2003-01-09)
   D7: THOMPSON R J ET AL: "STIMULATION OF A CHLAMYDOMONAS CHLOROPLAST PROMOTER BY NOVOBIOCIN IN-SITU AND IN

ESCHERICHIA-COLI IMPLIES REGULATION BY TORSIONAL STRESS IN THE CHLOROPLAST DNA" CELL, vol. 48, no. 2, 1987, pages 281-288.

D8: WO 01/02593 A (CALGENE LLC; MCBRIDE, KEVIN; OULMASSOV, TIM, N; MILLER, PAULA, C; ANDE) 11 January 2001 (2001-01-11)

3. Lack of novelty; article 33(2) PCT.

3.1 The documents D1-D2 and D4-D5 disclose a system for controlling the expression of a transgene in a plastid. This system implies the use (1) of a nuclear-encoded plastid-targeted phage RNA polymerase under the control of a (chemically) inducible promoter and (2) of a transgene integrated in the plastid genome under the control of a promoter specific for the phage RNA polymerase.

Due to the broad wording of the claims of the present application, the system disclosed in D1-D2 and D4-D5 will fit the definition of claims 1-2, 6, 9-13, 26, 28, 30-35 and 41-42. Therefore, claims 1-2, 6, 9-13, 26, 28, 30-35 and 41-42 cannot be considered as novel in the sense of article 33(2) PCT.

3.2 The documents D3 and D6 disclose, inter alia, the use of an inducible promoter for controlling the expression of a transgene in the plastids (especially a light-inducible promoter). Due to the broad wording of the claims of the present application, the ISA is of the opinion that claims 1-4, 9, 26, 28-29, 33-37, 39 and 41-42 cannot be considered as novel in the sense of article 33(2) PCT.

3.3 The document D8 discloses a system for modulating the activity of a promoter comprising incorporating in said promoter one or more cis element(s) from the luxI promoter and expressing a receptor protein from the LuxR family of transcriptional regulators, which, upon binding an acetylated homoserine lactone compound, interacts with the lux box, modulating the activity of the promoter (Abstract). The use of the system in chloroplasts is disclosed (p. 2, lines 14-16; p. 3, line 30 to p. 4, line 16). For use in chloroplast, the LuxR family protein is either expressed in the nucleus and transported in the plastid or expressed in the plastid. Examples 2B discloses the generation of plastid

expression constructs, example 4B discloses plant plastid transformation and example 5B discloses transplastomic plants analysis.

In the light of D8, the subject-matter of claims 1-6, 9-11, 13, 19, 26, 28, 30-31, 33-39 and 41-42 cannot be considered as novel in the sense of article 33(2) PCT.

4. Lack of inventive step; article 33(3) PCT.

4.1 The document D5 discloses the transformation of plastids with diverse constructs. The use of transgenes under the control of inducible promoters is disclosed even if no specific promoter is cited.
Light-inducible plastids promoters were known (D3 and D6) and D8 discloses the use of a chemically inducible control system in plastids.

The problem to be solved by the present application can be seen as the provision of an alternative method for controlling the expression of a transgene in plant plastids.

At the date of priority of the present application, it was well-known that prokaryotic promoters could function in plastids and vice versa due to the prokaryotic origin of plastids. Therefore, the ISA is of the opinion that the skilled person would have needed no inventive activity to contemplate using well-known prokaryotic systems like the (IPTG + lac repressor) or (Tetracyclin + tet repressor) systems in plastids.

Therefore, the subject-matter of claims 7-8 and 40 cannot be considered as inventive in the sense of article 33(3) PCT.

4.2 Claim 14-17 refer to the introduction of the nucleic acid by a RNA viral vector, by Agrobacterium-mediated or by leaf infiltration or via a phytopathogen like Agrobacterium. This techniques of transformation of plants are well-known in the art. Therefore, the subject-matter of claims 14-17 cannot be considered as inventive (article 33(2) PCT)

Re Item VIII

Certain observations on the international application

1. As a general remark, the ISA is of the opinion that the wording of most of the claims of the present application is too vague.

2. Claim 1 of the present application lacks clarity for the following reasons :
   (i) It is not clear what "controlling" the expression of a sequence should precisely mean, what renders the scope of the claim unclear.
   (ii) It is not clear if the sequence of interest could be endogenous to the chloroplast. In said case, the scope of claim 1 could include naturally occurring plastid genes. Some light-inducible plastid genes are known. These genes will fall under the scope of claim 1.
   (iii) It is not clear what is meant by "source" of a chemical signal what renders the scope of the claim unclear. This remark also applies to claims 6, 10 and 30.
   (iv) The wording "intra-plastid component of the plastid protein expression machinery" is vague and unclear. This remark also applies to claim 3.
   (v) It is not clear if the "intra-plastid component" has to be native for the plastid or if it could be imported from the cytoplasm or any other cell compartment what renders the scope of the claim unclear.

3. Claim 5 refers to "said recombinant nucleic acid". It is not clear which nucleic acid is referred to.

4. In claim 20, it is not clear what a "translation regulatory RNA" should precisely be what renders the scope of the claim unclear.

   This remark also apply to claims 22-25.

5. Claim 30 covers both proteinaceous and non-proteinaceous chemical signals. Claim 25 is, therefore, redundant with the claims referring to chemical signals in general terms.

6. The present application covers a huge number of different systems. However, the

present application only exemplifies a small number of such systems. The ISA is of the opinion that the skilled person would not be able to apply the teaching of the present application to other systems without undue burden of experimentation. Therefore, the claims of the present application can only be considered as supported by the description of the present application as far as they refer to the specific systems disclosed in the examples of the present application (see also point VIII-1 above) (article 5 PCT in combination with article 6 PCT).